CODING FORM FOR SRC INDEXING REVISED 10/15/86

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|---|---|---|
| Microfiche No. | | |
| OTS0515143 | | |
| New Doc I.D. | Old Doc I.D. | |
| 86-870000616 | | |
| Date Produced | Date Recieved | TSCA section |
| 8/15/81 | 7/27/87 | 8D |
| Submitting Organization | | |
| INTL ISOCYANATE INST | | |
| Contractor | | |
| UNIV OF TSUKUBA | | |
| Document Title | | |
| ACCUMULATION AND TOXICITY OF ISOCYANATES AND THEIR WATER REACTION PRODUCTS IN FISH WITH ATTACHMENTS AND COVER LETTER DATED 072287 | | |
| Chemical Category | | |
| TOLUENE DIISOCYANATE (1321-38-6) | | |

INTERNATIONAL ISOCYANATE INSTITUTE, INC.
119 CHERRY HILL ROAD
PARSIPPANY, NEW JERSEY 07054

TELEX 36[illegible]

CONTAINS NO CBI



(201) 263-7517



22 July 1987

86-870000616

Document Processing Center (TS-790)
Office of Toxic Substances
Environmental Protection Agency
401 M Street, S.W.
Washington, D. C. 20460

Attention: 8(d) HEALTH and SAFETY REPORTING RULE (REPORTING)
May 1, 1987

Dear Sir or :

As described at 40 C.F.R. 716.20(a) (10), the International Isocyanate Institute (III) submits the enclosed studies on behalf of its members to satisfy member reporting requirements under Section 8(d) of the Toxic Substances Control Act. These studies are on chemicals added to the 8(d) list on May 1, 1987. The studies are indexed by CAS numbers with chemical name, III identification number and title provided.

Attachment #1 is an indexed list of completed studies.

Attachment #2 is a compilation of the reports from the completed studies.

Attachment #3 is an indexed list of studies that are currently in progress.

Please refer to the III identification number in any communication regarding the report.

If the Agency needs further information, please do not hesitate to contact me.

Very truly yours,

R. K. Rigger
Managing Director

RKR/c
enclosures

86-870000616

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 101-68-8 Benzene, 1,1'-methylenebis[4-isocyanato-
Methylenedi-p-phenylene diisocyanate
4,4'-Methylenebis(phenyl isocyanate)
MDI
4,4'-Diisocyanatodiphenylmethane

| III NUMBER | TITLE |
|---|---|
| 10000 | Prepolymeric MDI (Biphenylmethane Diisocyanat) with and without added Phenyl Isocyanate (PhI) - one hour acute inhalation toxicity. |
| 10005 | Determination of the concentration of vapor generated from monomeric 4.4'-Diphenylmethane Diisocyanate (MDI) by a dynamic method. |
| 10008 | Two-day study into the relation between polymeric MDI concentration values obtained by a QCM-Cascade, HPLC and Colorimetry. |
| 10010 | Liquid Waste after TDI/MDI decontamination. |
| 10012 | Literature Study on Reaction of Isocyanates with Biological Materials. |
| 10013 | Report on fire hazard of Isocyanate chemicals. |
| 10014 | Report on fire hazard of Isocyanate chemicals. |
| 10018 | Analytical methods to monitor aerosols of Polymeric 4,4'-Diphenylmethane-diisocyanate (MDI) at low concentrations. |
| 10019 | Aquatic life study phase II, step 2 Accumulation of TDI, MDI, TDA and MDA in fish and their toxicity. |
| 10022 | Generation and monitoring of breathable aerosols of polymeric 4,4'-diphenylmethane-diisocyanate (MDI). |

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS #101-68-8 Benzene, 1,1'-methylenebis[4-isocyanato-
Methylenedi-p-phenylene diisocyanate
4,4'-Methylenebis(phenyl isocyanate)
MDI
4,4'-Diisocyanatodiphenylmethane

| III NUMBER | TITLE |
|---|---|
| 10026 | Pre-polymeric diphenylmethane,4,4', diisocyanate (Petmar MDI)<br>Pre-polymeric diphenylmethane,4,4', diisocyanate + phenyl isocyanate. 50 ppm.<br>Pre-polymeric diphenylmethane,4,4', diisocyanate + phenyl isocyanate. 150 ppm.<br>An experiment to investigate the relative sub-acute toxicity of the above substances in the rat by inhalation. |
| 10050 | Metabolism and toxicogenetics of Methylenedianiline. |
| 10065 | A study of the diffusion of MDI in rats contaminated via the respiratory system. |
| 10074 | Investigations on the microbial degradation of PU forams. Part II. |
| 10075 | Respiratory Sensitivity Study. |
| 10076 | Deposition of aerosol components on the hair of rats exposed to polymeric MDI aerosols. |
| 10077 | Acute inhalation toxicity study of polymeric MDI in rats. |
| 10092 | Biological action of TDI and MDI in water. |
| 10129 | Immunological aspects of Isocyanates. |
| 10187 | Isocyanates : Irritation and Hypersensitivity. |
| 10188 | Preliminary study on skin sensitization caused by MDI solutions. |

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 101-68-8 Benzene, 1,1'-methylenebis[4-isocyanato-
Methylenedi-p-phenylene diisocyanate
4,4'-Methylenebis(phenyl isocyanate)
MDI
4,4'-Diisocyanatodiphenylmethane

| III NUMBER | TITLE |
|---|---|
| 10206 | Aquatic life study Phase II, Step 2, Accumulation of TDI, MDI and their reaction products in Daphnia. |
| 10223 | TDI and MDI immunological studies. Summary report of research supported by the International Isocyanate Institute. |
| 10234 | Aquatic life study Phase II, Step 1. Biodegradation of TDI and MDI in the model river and marine water. |
| 10243 | Mortality among workers exposed to isocyanates. Feasibility Study. |
| 10253 | Sub-chronic (13 week) inhalation toxicity study of polymeric MDI aerosol in rats (part B2) |
| 10258 | Ecotoxicity of Toluenediisocyanate (TDI) Diphenylmethanediisocyanate (MDI) Toluenediamine (TDA) Diphenylmethanediamine (MDA) |
| 10299 | Aquatic Life Studies |
| 10317 | Production and control of breathable MDI aerosols for primal experiments. |

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 101-68-8 Benzene, 1,1'-methylenebis[4-isocyanato-
Methylenedi-p-phenylene diisocyanate
4,4'-Methylenebis(phenyl isocyanate)
MDI
4,4'-Diisocyanatodiphenylmethane

| III NUMBER | TITLE |
|---|---|
| 10360 | Generation of 4,4' Diphenylmethane Diisocyanate (MDI) vapour |
| 10386 | Pharmacokinetics of MDI after inhalation exposure of rats to labelled MDI. |
| 10391 | Skin sensitization by isocyanates. |
| 10393 | Study of the burning characteristics of isocyanate chemicals. |
| 10439 | Di-Isocyanate Induced Asthma - Reactions to TDI, MDI, HDI and Hisamine. |
| 24298 | Acute Inhalation Toxicity ($LC_{50}$) in the Male Albino Rat. |

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS #1321-38-6 Benzene, diisocyanatomethyl-(unspecified isomer)

| III NUMBER | TITLE |
|---|---|
| 10010 | Liquid waste after TDI/MDI decontamination. |
| 10012 | Literature Study on Reaction of Isocyanates with Biological Materials. |
| 10013 | Report on fire hazard of Isocyanate chemicals. |
| 10014 | Report on fire hazard of Isocyanate chemicals. |
| 10019 | Aquatic life study phase II, step 2 Accumulation of TDI, MDI, TDA and MDA in fish and their toxicity. |
| 10024 | Tolylene di-isocyanate three week inhalation toxicity in the rat. |
| 10033 | Stack Emission Part B : Emitted TDI Gas Treatment with Activated Carbon. |
| 10034 | Stack Emission Part A : Emitted TDI Gas Treatment with Activated Sludge. |
| 10035 | The toxicity and carcinogenicity to rats of Toluene Diisocyanate vapour administered by inhalation for a period of 113 weeks. |
| 10040 | Reaction of TDI with water and with wet sand. |
| 10044 | Emission of Tolylene Diisocyanate (TDI) and Tolylene Diamine (TDA) in flexible polyurethane foam production lines. |
| 10045 | Emission of Tolylene Diisocyanate (TDI) and amines. |
| 10055 | Preparation and evaluation of a system for exposing rats to Toluene Diisocyanate vapour. |

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

| III NUMBER | TITLE |
|---|---|
| 10057 | Evaluation of a system for exposing hamsters to Toluene Diisocyanate vapour. |
| 10064 | A study of the diffusion rate of TDI in rats contaminated via the respiratory system. |
| 10074 | Investigations on the microbial degradation of PU foams. Part II |
| 10075 | Respiratory sensitivity study. |
| 10089 | Studies of Toluene Diisocyanate induced pulmonary disease. |
| 10092 | Biological action of TDI and MDI in water. |
| 10094 | Foam plant stack emission data. |
| 10095 | Stack Emission Part 3 : Emitted TDI Gas Treatment with Activated Carbon "Regeneration of Spent Activated Carbon". |
| 10096 | Stack Emission Part A : Emitted TDI Gas Treatment with Activated Sludge. |
| 10098 | Epidemiological study for effects of TDI. |
| 10100 | Histopathological observations on selected tissues of syrian hamsters exposed by inhalation to vapors of Toluene Diisocyanate (TDI) for 6 hours/day, 5 days/week for 4 weeks. |
| 10116 | Review of the incidence of rhinitis in rats exposed chronically to Toluene Diisocyanate vapour. |

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

| III NUMBER | TITLE |
|---|---|
| 10117 | Review of the national toxicology program carcinogenesis bioassay of Toluene Diisocyanate. |
| 10121 | Toluene Diisocyanate (TDI) proposed exposure standard. |
| 10129 | Immunological aspects of Isocyanates. |
| 10142 | Toluene Diisocyanate acute inhalation toxicity in the rat. |
| 10153 | A 30-day repeated inhalation toxicity study of Toluene Diisocyanate (TDI) in laboratory animals. |
| 10159 | The fate of Toluene Diisocyanate. |
| 10162 | Epidemiological study for effects of TDI. |
| 10163 | Validation of MCM 4000 personal monitor and MCM 4100 integrating reader/recorder system. |
| 10168 | Summary of work carried out on FE-A-14 III - 1 by H. Sakurai and co-workers. |
| 10169 | The toxicity and carcinogenicity to rats of Toluene Diisocyanate vapour administered by inhalation for a period of 113 weeks. |
| 10175 | Emission of Tolylene Diisocyanate (TDI) and Tolylene Diamine (TDA) in flexible polyurethane foam production lines. |
| 10184 | Immunological studies on TDI exposed workers. Part I. |
| 10187 | Isocyanates : Irritation and Hypersensitivity. |

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

| III NUMBER | TITLE |
|---|---|
| 10206 | Aquatic life study Phase II, Step 2, Accumulation of TDI, MDI and their reaction products in Daphnia. |
| 10208 | The Toxicity and Carcinogenicity to rats of Toluene Diisocyanate vapour administered by inhalation for a period of 113 weeks. Addendum Report. Vol. 2. |
| 10210 | The Toxicity and Carcinogenicity to rats of Toluene Diisocyanate vapour administered by inhalation for a period of 113 weeks. Vol. I |
| 10223 | TDI and MDI immunological studies. Summary report of research supported by the International Isocyanate Institute. |
| 10233 | The Toxicity and Carcinogenicity to rats of Toluene Diisocyanate vapour administered by inhalation for a period of 113 weeks. Addendum Report. Vol. 1 |
| 10234 | Aquatic life study Phase II, Step 1. Biodegradation of TDI and MDI in the model river and marine water. |
| 10237 | Isocyanate monomer in PU foam. |
| 10243 | Mortality among workers exposed to isocyanates. Feasibility Study. |
| 10258 | Ecotoxicity of Toluenediisocyanate (TDI). Diphenylmethanediisocyanate (MDI) Toluenediamine (TDA). Diphenylmethanediamine (MDA) |
| 10259 | Sampling and Analysis of TDI atmospheres at Klinikum Grosshadern, Munich. |

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

| III NUMBER | TITLE |
|---|---|
| 10299 | Aquatic Life Studies. |
| 10307 | Studies on the effects of TDI on living animals. |
| 10308 | Change of TDI in olive oil. |
| 10321 | Improvement in RAST for TDI. Parts A and B. |
| 10340 | Audit of the national toxicology program carcinogenesis bioassay of toluene diisocyanate. |
| 10345 | Isocyanate spillage control. |
| 10348 | Immunological Studies on TDI exposed workers Part II. |
| 10349 | Isocyanate hypersensitivity. |
| 10382 | The toxicity and carcinogenicity of Toluene Diisocyanate vapour when administered to mice over a period of approximately 2 years. Summary Report. |
| 10383 | The toxicity and carcinogenicity of Toluene Diisocyanate vapour when administered to mice over a period of approximately 2 years. |
| 10391 | Skin sensitization by isocyanates. |
| 10393 | Study of the burning characteristics of isocyanate chemicals. |

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS #1321-38-6 Benzene, diisocyanatomethyl-(unspecified isomer)

| III NUMBER | TITLE |
|---|---|
| 10416 | Sampling and analysis of TDI atmospheres at Klinikum Grosshadern, Munich. |
| 10430 | Protective effect of drugs on late asthmatic reactions and increased airway responsiveness induced by Toluene Diisocyanate in sensitized subjects. |
| 10433 | The reactions of OH radicals with Toluene Diisocyanate, Toluenediamine, and Methylene Dianiline under simulated atmospheric conditions. |
| 10434 | Metabolism and disposition of $^{14}C$-labeled Toluene Diisocyanate (TDI) following oral and inhalation exposure ; Preliminary studies. |
| 10437 | Toluene Diisocyanate-Induced Asthma: Bronchial Provocation and Reactivity Studies. |
| 10438 | Toluene Diisocyanate-Induced Asthma: Inhalation Challenge Tests and Bronchial Reactivity Studies. |
| 10439 | Di-Isocyanate Induced Asthma- Reactions to TDI, MDI, HDI and Histamine. |

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 91-08-07 Benzene, 1,3-diisocyanato-2-methyl
TDI, 2-6-diisocyanate

| III NUMBER | TITLE |
| --- | --- |
| 24207 | Disposition of 2,6-Toluene Diisocyanate in Fischer 344 rats |

ATTACHMENT #2

COMPILATION OF REPORTS FROM III FILES
(AS INDEXED IN ATTACHMENT #1)

These reports are in envelopes labeled Attachment #2 and are packaged, along with an envelope, addressed to:

Document Processing Center (TS-790)
Office of Toxic Substances
Environmental Protection Agency
401 M Street, S.W.
Washington, D. C. 20460

Attention: 8(d) HEALTH and SAFETY REPORTING RULE
(REPORTING) May 1, 1987

from:

International Isocyanate Institute, Inc.
119 Cherry Hill Road
Parsippany, New Jersey 07054

containing a transmittal letter for these documents.

ATTACHMENT #3

INDEXED LIST OF STUDIES IN PROGRESS

CAS # 101-68-8 Benzene, 1,1'-methylenebis[4-isocyanato-
Methylenedi-p-phenylene diisocyanate
4,4'-Methylenebis (phenyl isocyanate)
MDI
4,4'-Diisocyanatodiphenylmethane

| III NUMBER | TITLE |
|---|---|
| E-A-8 | Study of chronic toxicity and carcinogenicity of polymeric MDI aerosol in rats. Part C Study. |

Current work authorized to begin June 1985.
To study chronic toxicity and carcinogenicity of polymeric MDI aerosol in rats. Data sought - Effect on animal tissues.
Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time.
CIVO Institution, Tno., Toxicology and Nutrition, Utrechtsewe 848, P.O. Box 306, 3700 A.J. Zeist, The Netherlands.

| | |
|---|---|
| E-H-44 | MDI sampling and analysis at CIVO |

Current work authorized to begin November 1984.
To study consistency/comparability of various methods continuous/discontinuous for determining the composition of atmospheres in Study E-A-8 (Part C) above. Data sought - Analytical data on polymeric MDI aerosol atmospheres.
Our current estimated completion date for this study is the first quarter 1989. It may be possible to complete this study before 1989; however, it may require more time.
CIVO Institution, Tno., Toxicology and Nutrition, Utrechtsewe 848, P.O. Box 306, 3700 A.J. Zeist, The Netherlands.

ATTACHMENT #3

INDEXED LIST OF STUDIES IN PROGRESS

CAS # 1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

| III NUMBER | TITLE |
|---|---|
| E-B-11 | Epidemiological study of workers in U.K. flexible foam industries. |

Current work authorized to begin Mid 1978.
To investigate whether working on flexible PU foam manufacturing plants gives rise to increased expectation of decrements in lung parameters above those due to ageing.
Data sought - monitoring of exposed workers' and controls' lung parameters. Monitoring of airborne TDI (and on limited scale of tertiary aliphatic amine) in the workplace.
Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time.
Tynestead Limited, Tynestead House, 22 Camberley Drive, Bamford, Rochdale, Lancs, OL11 4 AZ, UK. and Medical Research Council, 20 Park Crescent, London, UK.

ATTACHMENT #3

INDEXED LIST OF STUDIES IN PROGRESS

CAS # 1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

| III NUMBER | TITLE |
|---|---|
| FE-AB-14 | Epidemiological study of workers in Japan flexible foam industries. Phase V. |

Current work authorized to begin August 1985.
To clarify relationship between TDI concentration and chronological change in pulmonary and respiratory symptoms of workers in PU foam plants. Data sought.
Monitoring of exposed workers' and controls' lung parameters.
Monitoring of airborne TDI in the workplace.
Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time.
School of Medicine, Keio University, Shinjuki-Ku, Tokyo, Japan.

ATTACHMENT # 3

INDEXED LIST OF STUDIES IN PROGRESS

CAS # 1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

| III NUMBER | TITLE |
|---|---|
| E-E-22 | Clean Stack Air Project<br><br>Current work authorized to begin March 1980.<br>To study ways in which TDI Emissions from flexible foam plants can be removed from exhaust gases by carbon absorption.<br>Data sought - Concentrations of TDI at inlets and outlets of carbon absorption units.<br>Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time.<br>Dunlop (Now BTR, Silvertown House, Vincent Square, London, UK. |
| E-AB-40 | An investigation into the mortality and cancer morbidity of production workers in the UK flexible polyurethane foam industry.<br><br>Current work authorized to begin July 1987.<br>To compare the mortality and cancer morbidity experience of production workers in UK flexible foam manufacturing plants with those of unexposed controls and of the population at large, and to determine, if appropriate, possible reasons for differing experiences.<br>Data sought.<br>Comparative Data on death and illness due to cancer, analysed statistically. Data sought.<br>The expected date of termination of project is indeterminate since it depends on results found at different intervals. The first analysis will take place 1989.<br>Cancer Epidemiology Unit, University of Birmingham, Edgbaston, Birmingham UK. |

ATTACHMENT #3

INDEXED LIST OF STUDIES IN PROGRESS

CAS #1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

| III NUMBER | TITLE |
|---|---|
| NA-E-24 | Fate of airborn TDI (Part II) |

Current work authorized to begin May 1984.
To determine the fate of airborne TDI and the effects of moisture, light, and atmospheric pollutants on TDI loss from the gas phase.
Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time.
Battelle Columbus Laboratories, 505 King Avenue, Columbus, Ohio 43201

NA-AB-26 Detecting delayed isocyanate sensitivity.

Current work authorized to begin May 1, 1987.
This research is being conducted to better detect delayed isocyanate sensitivity in persons exposed and/or sensitized to isocyanates. In 1986, M. Karol's work was directed towards identification of isocyanate-specific lymphocytes by class.
Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time.
Dr. M. Karol, University of Pittsburgh, 130 Desoto Street, Pittsburgh, Pennsylvania 15261

ATTACHMENT #3

INDEXED LIST OF STUDIES IN PROGRESS

CAS #1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

| III NUMBER | TITLE |
|---|---|
| NA-AB-43 | Improvement of RAST tests for TDI |

Current work authorized to begin May 1, 1987.
This research is being conducted to improve RAST (Radiolabeled Antibody Sorbent Technique) test for identifying exposure and sensitization to TDI. Additional mechanistic work on TDI sensitization is being conducted by Dr Brown. This includes studying proteins in TDI exposed animals.
Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time.
Dr W. E. Brown, Carnegie-Mellon University, Pittsburgh, Pa. 15261.

NA-AB-50 TDI Reprotoxicity

The teratology study was initiated in the 4th quarter of 1986.
The reproduction study was initiated in the 2nd quarter of 1987.
This project evaluates both the "Developmental Toxicity of Inhaled TDI in CD (Sprague-Dawley) Rats" and "Two-Generation Reproduction Toxicity of TDI in CD (Sprague-Dawley) Rats."
Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time.
Dr T. W. Tyl, Bushy Run Research Center, RD #4, Mellon Road, Export, Pennsylvania 15632.

Master

10019

86-870000616

III Contract No: FE-E-19-II-2

Date of Report: August 15, 1981

Complete Report to

The International Isocyarate Institute

by

Institute of Community Medicine

The University of Tsukuba

# Aquatic Life Study Phase II, Step 2

# Accumlation of TDI, MDI, TDA and MDA in Fish and their Toxicity.

Report prepared by: Prof. Dr. Kikuo Fujiwara

CONTENTS

## 1. INDEX of TABLES, FIGURES and PHOTOGRAPHS

Photograph 11 — Bone tissue of carp lived for 35 days in river water added 10 ppm of TDI

Photograph 12 -- Bone tissue of carp lived for 35 days in river water added 10 ppm of MDI

## 2. INTRODUCTION

The environmental contamination with isocyanates may produce the possibilities of impact upon aquatic life system, however, the studies concerning the accumulation of these chemicals and their water reaction products in fish bodies are not reported as yet. Furthermore, their toxic effect on bacterial cells in water have not been investigated quantitatively. And besides, various poisonous influences of these substances in fish bodies have not been examined pathologically as yet.

The systematic investigations about these problems are necessary for environmental safety control. The present study deals with the hazardous properties of the isocyanates and their water reaction products experimentally.

## 3. OBJECTIVES

In this investigation, accumulation and toxicity of the isocyanates and their water reaction products in fish bodies are tested using river model and the tissue materials from the fishes exposed to the chemicals are examined histopathologically. Furthermore, the poisonous effects of these substances on the bacterial cells in water are investigated in view of the biological chains.

## 4. SUMMARY and CONCLUSIONS

In the accumulation tests of TDI, MDI and their water reaction products in carp, the isocyanates and their amines could not be detected in the whole bodies of the test fishes having lived for 8 weeks in the river model and besides, these chemicals were all negative in the kidneys from these fishes. Consequently the accumulation of these isocyanates and their amines is regarded as negative in fish bodies.

The bacteriocidal effects of TDA against living cells of E.coli were evidenced in the saline solutions, however, TDI, MDI, TDA and MDA did not inhibit the growth of the same bacterial cells in the nutrient broth at 37°C when they were added at the concentration of 100 ppm and 50 ppm respectively.

TDI and MDI killed fishes (orange-red killifish and carp) when they were added at the concentration of 10 ppm in the river model and prior to death they showed collision to the wall of the river model.

In the gross examination of the test carps, skin tissues fell off and anal haemorrhages were observed. The brownish pigmentation was remarkable in the muscular tissues of the necropsy cases. Edema and infiltration of the lymphocytes were evidenced in the interstitial tissues of the liver and kidney histopathologically.

## 5. DISCUSSION

The environmental toxicity of the isocyanates in the air has been investigated from the viewpoint of the industrial hygiene[1),2)]. However, their hazardous properties for aquatic life have not been examined in details as yet, though it is necessary to have enough informations concerning the toxic effects on the living things in water to prepare proper procedures in the field of general public health.

The isocyanates and their amines were not accumulated in the body of fishes in the river model experimentally in this study. Consequently, it may be unnecessary to feel anxiety about the risk of poisoning in human beings and other animals through food chains.

Although the isocyanates can be decomposed rapidly when they are spilt in aquatic environments as shown in our previous report[4)], their water reaction products have possibilities to harm many species of the aquatic life. This study revealed the bacteriocidal effect of TDA on E.coli in the physiological saline solution and other pathogenic activities against fishes in the river model. These toxic properties are regarded as an inflammatory irritants from the results of the histopathological examination with fish bodies.

## 6. DETAILED EXPERIMENTAL RESULTS

### 6-1. Accumulation tests of TDI, MDI and their reaction products in fishes

The isocyanates and their amines were tested for their accumulation in fishes in the river model outdoors.

6-1-1 Material tested:---

TDI: TDI 80 Mitsubishi Chemical Industries, Ltd.

MDI: Polymeric isocyanates, Sumidur 44v-20 Sumitomo Bayer Urethane Co., Ltd. herein after refered to MDI.

Test fish: Carp (Cyprinus Carpio) weighing 30 g, about 10 cm in body length.

6-1-2 River model:---

As a simulation experiment of accumulation, a system of a river model was used and operated outdoors in order to test the natural factors. The river wall was made with stainless steel and the river water flowed back through an electric circulator at the rate of 10 cm per second on the average.

Lines: 2 lines; for test chemical and for blank (no chemical).

Length: 5 m, Breadth: 0.5 m, Depth: 1 m.

Circulator: 200 l/min.

Flow rate: 4-14 cm/sec.

6-1-3 Water used:---

Natural river water containing bottom sludge at the rate of 0.5% v/v.

6-1-4 General procedure:---

The isocyanates were added to the water in the river model at the concentration of 0.1 ppm respectively and mixed vigorously with a stick. After mixing, the system of circulation worked and water flowed continuously for 8 weeks. The temperature of the river water was about 23°C-25°C. The carps were kept in a wire netting basket set in the river model and the pelleted feed was given to the fishes during the experiment. Sample fishes were taken from the basket and kidneys were removed. The samples and other parts of bodies of the test fishes were homogenized separately with a mixing blender. These homogenates were used for determination of the isocyanates and their amines.

6-1-5 Analyticals:---

After removal of the protein components from the homogenates of fish bodies with trichloroacetic acid (Final concentration : 5%), the isocyanates and their amines were determined according to the Domsch's method[3] modified by Fujiwara[4]. As the control samples the isocyanates and their amines were added to the homogenates of untreated fishes artificially and the contents of the additives were determined with the method above mentioned. The recovery rates of the isocyanates and their amines were 88-92% in the determination.

6-1-6 Experimental results:---

After feeding for 8 weeks in the river model added with TDI, four fishes were tested for determination of TDI and TDA and they were all negative in both quantitative experiments.

After feeding for 8 weeks in the river model added with MDI, four fishes were examined to determine MDI and MDA and they were also all negative results. The minimum limits of the concentration for determination of these isocyanates and amines by this method are less than 0.1 ppm. Consequently, it is concluded that these isocyanates and their amines can not be accumulated in fish body.

6-2. Changes in viable counts of bacteria due to TDI, MDI and their amines.

Bacteriocidal effects of isocyanates and their amines were tested with E.coli in the physiological saline solution and the inhibitive activities of these compounds in growth of the same species of bacteria were examined in broth.

6-2-1 Materials tested:——

TDI, MDI: same samples as used in Experiment 6-1.

TDA: 2, 4-Diaminotoluene, Tokyo Kasei Co., Ltd. extra-pure.

Bacterial strain tested: Escherichia coli No. 3284 (Type culture in The Research Institute for Chemobiodynamics, Chiba University, Japan).

Nutrient broth: Commercial product of Eiken Kagaku Co., Ltd.

Desoxycholate agar: Commercial product of Nissui Co., Ltd.

6-2-2 Culture medium:——

Nutrient broth: 3 g of meat extract, 10 g of peptons and 5 g of sodium chloride are dissolved in 1 liter of water and the

solution is adjusted to pH 7.0. The nutrient broth is sterilized in an autoclave at 121°C, 15 min. The physiological saline solution is also sterilized by the same procedure. Desoxycholate agar: 1 g of sodium desoxycholate, 10 g of peptons, 2 g of ferric ammonium citrate, 5 g of sodium chloride, 2 g of monobasic potassium phosphate, 10 g of lactose, 0.033 g of neutral red and 15 g of agar are added into 1 liter of water and dissolved by heating. The agar medium is poured into the petri dishes without sterilization.

6-2-3 General procedure:---

The inoculum culture of the bacteria is prepared with nutrient broth by incubation for 18 hours at 37°C. In the test of bacteriocidal activities, the inoculum was added into the physiological saline solution ($10^6$/ml) containing TDA and MDA respectively at the concentration of 100 ppm and 50 ppm. Samples are taken from the solution seriatim and the viable counts are performed with the plates of desoxycholate agar. The examination of the inhibitive properties in the growth of bacterial cells consists of viable counts after incubation of inoculum in the nutrient broth containing isocyanates and their amines at the concentration of 100 ppm and 50 ppm respectively by shaking culture at 25°C.

6-2-4 Viable count:---

The numbers of the viable counts are obtained from the results of three plates for each sample. The plate count agar are prepared by the procedure of three layers method.

6-2-5 Experimental results:---

TDA and MDA showed the bacteriocidal activities against the living cells in the physiological solutions in a few days (Figure 1). However, they could not inhibit the growth of the bacteria in the nutrient broth. TDI and MDI did not inactivate the growth of the same culture even at the concentration of 100 ppm remarkably (Figure 2, 3).

6-3. Killing effect of TDI, MDI on fishes

As a simulation experiment, the killing effects of the isocyanates were examined for orange-red killifish and carp in the river model by feeding test for 35 days.

6-3-1 Materials tested:---

TDI, MDI: same samples as used in Experiment 6-1.

Orange-red killifish: Orizias latipes

Carp: Cyprinus carpio, same fishes as used in Experiment 6-1.

6-3-2 River model:---

Same system of the river model used in Experiment 6-1.

6-3-3 Water used:---

Natural river water containing sludge same as in Experiment 6-1.

6-3-4 General procedure:---

As a preliminary test, nine orange-red killifishes were exposed to TDI at the concentration of 50 ppm. In the 10 ppm group of the experiment, 10 killifishes were used. In

the tests of carps, they were fed in the river model added with 10 ppm of TDI and MDI respectively for 35 days.

6-3-5 Experimental results:---

In the 50 ppm test group, all fishes died within 24 hours. In the 10 ppm of TDI concentration, all of ten killifishes died in 33 days (Table 1).

In the carp experiments, they were killed by TDI and MDI as Table 2, however, one fish survived for 35 days in each isocyanate group and was sacrificed for further examination.

6-4. Pathological examination of liver, kidney and bone in carp bodies exposed to the isocyanates were examined morphologically. During feeding in the river model, the behaviour of the fishes tested were observed and it was found that they came to collision with walls repeatedly. This phemomenon might be originated from the pathological changes in brain tissues which were evidenced by the histological examination, i.e. remarkable edema formation. LeQuesne reported the neurological complications due to TDI in human beings in details.

6-4-1 Materials tested:---

The liver, kidney and bone samples were isolated from fish bodies survived for 35 days in the river model added with 10 ppm of the isocyanates. Prior to the preparation of these organs the general features of the viscera were observed and photographed.

6-4-2 General procedure:---

The organs were fixed with formalin solution and stained by haematoxylin-eosin method excepting bone tissues. For the staining of the bone tissues Masson's method was applied.

6-4-3 Gross examination:---

The peeling-off of the skin tissues from the bodies of the fishes tested was observed. The muscle tissues showed brown pigmentation which was regarded as the reaction product from haemoglobin compounds (Photograph 2, 3). The congestion was evidenced in the muscular tissues histologically. Anal haemorrhage was evident.

6-4-4 Histopathological examination:---

Liver: Congestion and edema were observed in the interstitial tissues. Lymphocytes were infiltrated with other round cells into the tissues of liver. No state of necrosis and degeneration was found and no growth of elastic fiber was evidenced (Photograph 5, 6).

Kidney: No change was found in the tissues of the glomerulus and epidermis of the uriniferous tubule. The infiltration of the lymphocytes and neutrophyl leucocytes were observed in the interstitial tissues. General edematic feature was evident in the kidney (Photograph 8, 9).

Bone: No pathological change was detected in the tissues of bone from carps exposed to the isocyanates, comparing to the control sample (Photograph 11, 12).

## 7. LITERATURE


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3. Domsch, K. H. et al: Investigations into the formation of 2,4- and 2,6-toluylendiamines through breakdown of polyurethane flexible foam from the Cladosporium herbarum. SC/EE-12, p.7. 1976
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Table 1. Killing effect of TDI against Orange-red Killifish

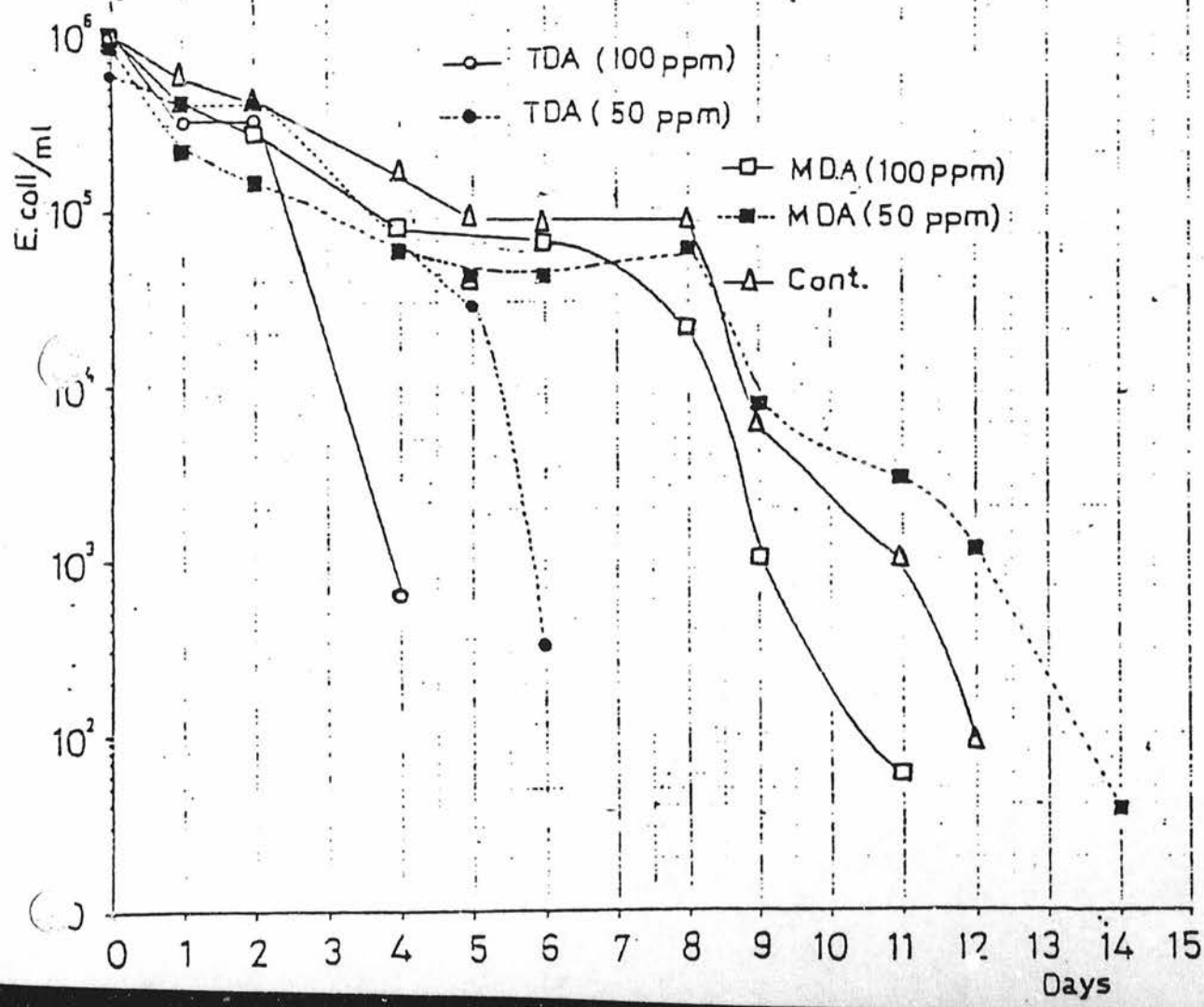
| Days of experiment | 16 | 20 | 23 | 29 | 30 | 32 | 33 |
|---|---|---|---|---|---|---|---|
| Number of fishes dies | 1 | 1 | 1 | 2 | 1 | 3 | 1 |

Number of fishes tested : 10
TDI concentration : 10 ppm

Table 2. Killing effect of TDI and MDI against carp

| Days of experiment | 5 | 7 | 10 | 14 | 15 | 20 | 35 |
|---|---|---|---|---|---|---|---|
| Number of fishes died | | | | | | | |
| TDI | 3 | 1 | | 4 | 1 | | 1 (sacrificed) |
| MDI | | | 1 | | 2 | 4 | 1 (sacrificed) |

Number of fishes tested : 10
Isocyanate concentration : 10 ppm


TDA (100 ppm)
TDA (50 ppm)
MDA (100 ppm)
MDA (50 ppm)
Cont.
E. coli/ml
$10^6$
$10^5$
$10^4$
$10^3$
$10^2$
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days

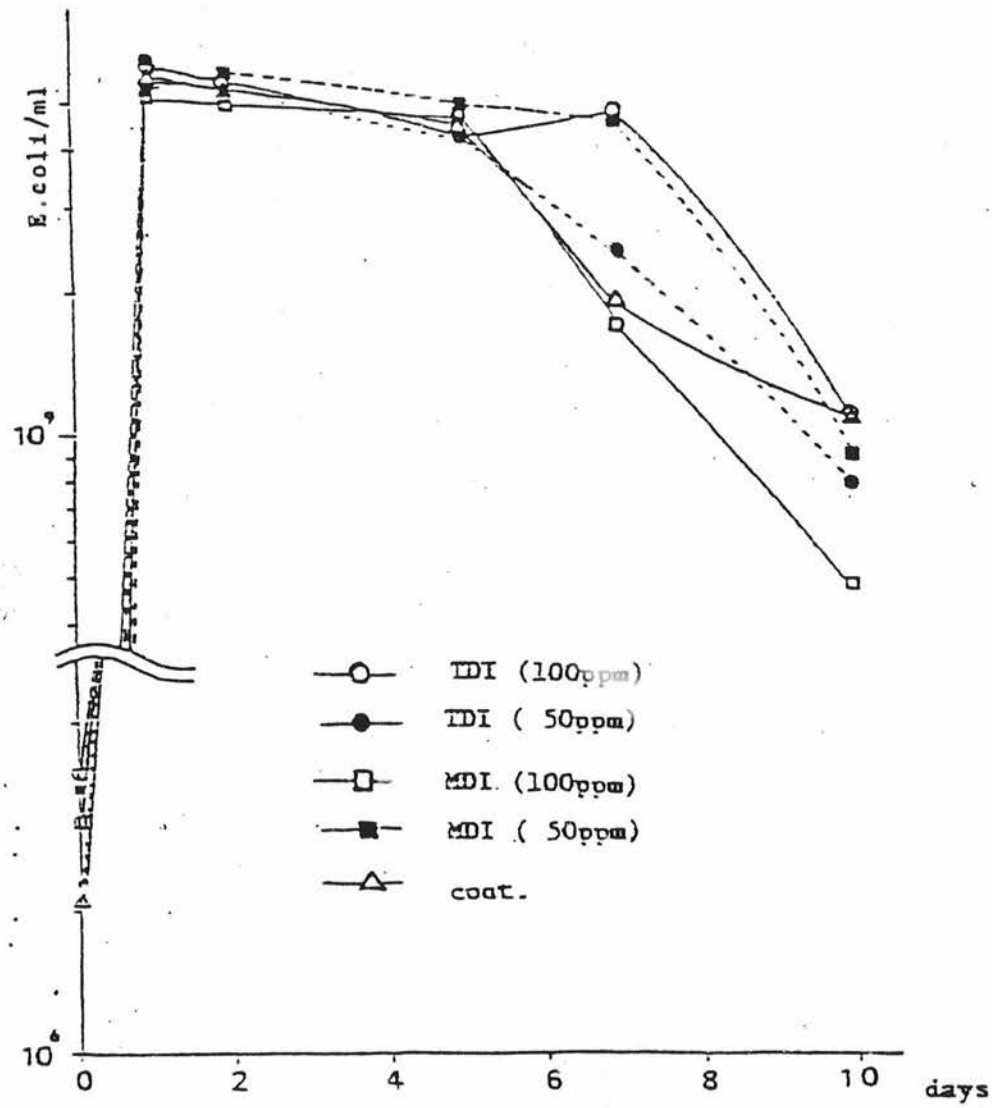



Fig. 2 Growth curve of E.coli in the nutrient broth added TDI and MDI

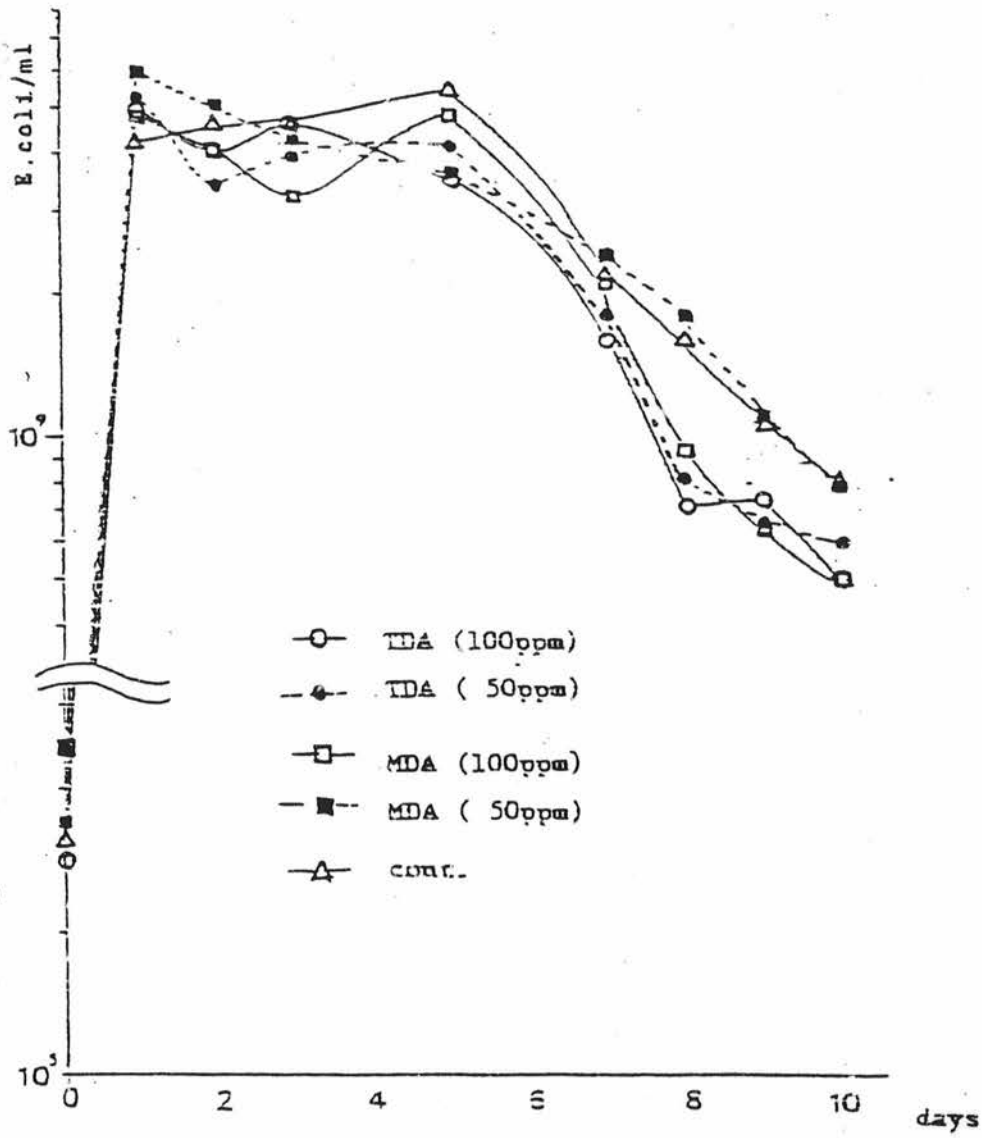
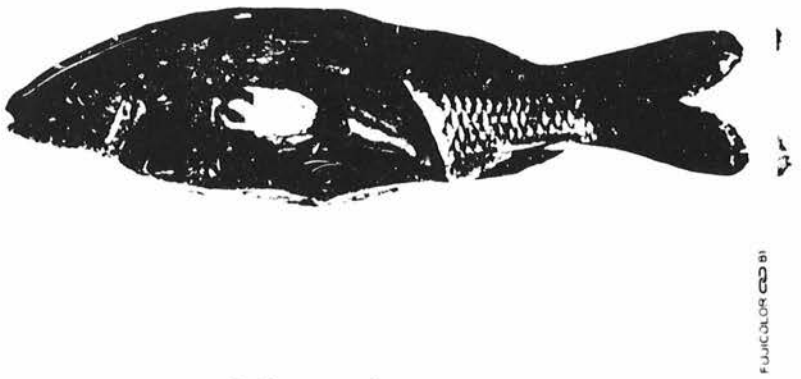



Fig. 3 Growth curve of E.coli in the nutrient broth added TDA and MDA


Photograph 1 —— Carp control

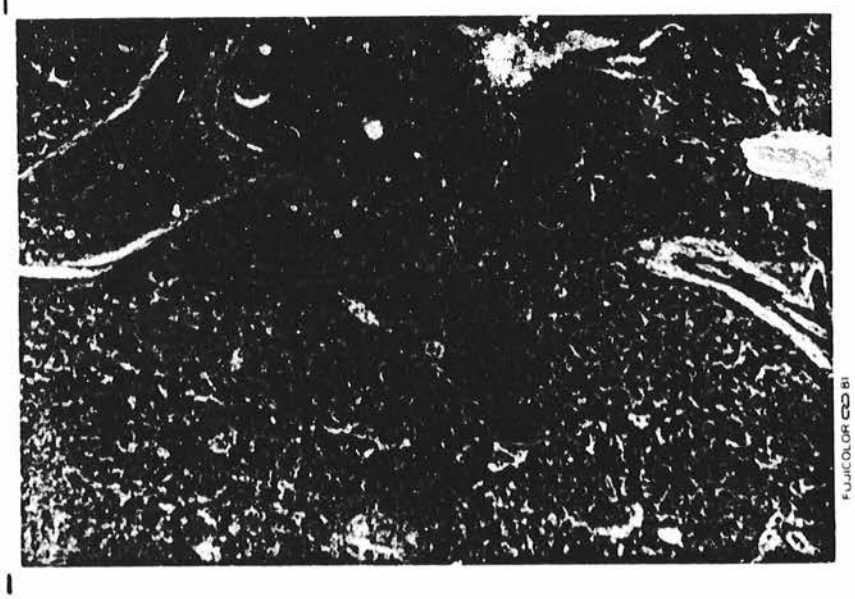

TDI 35 days

Photograph 2 —— Carp lived for 35 days in river water added 1[illegible] ppm of TDI

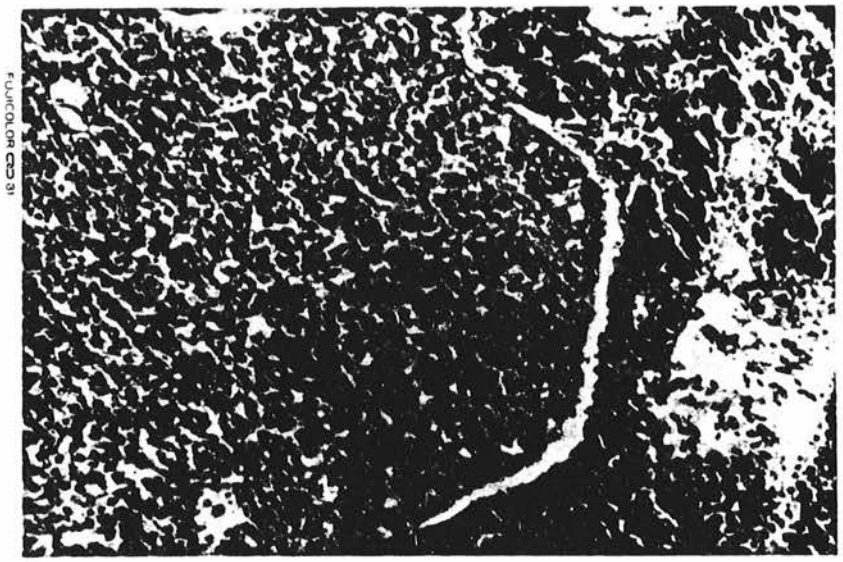


MDI 35 days

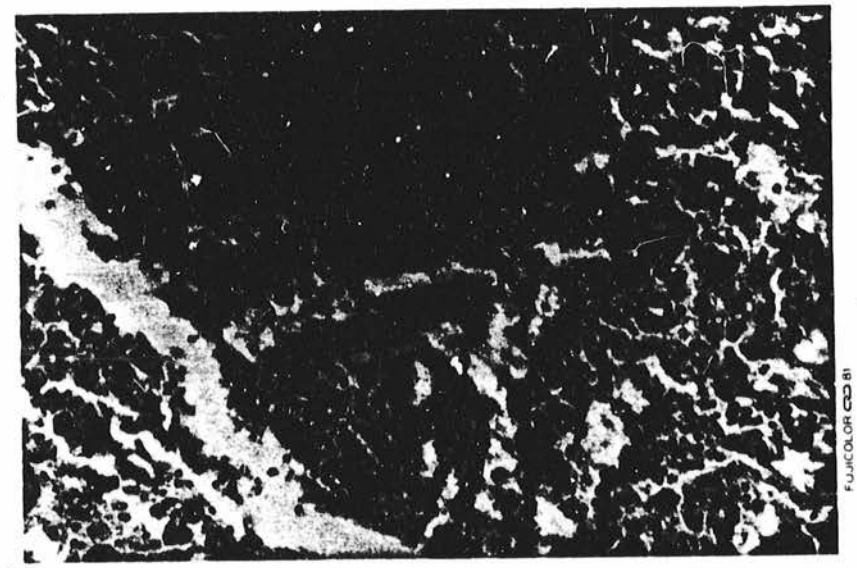
Photograph 3 —— Carp lived for 35 days in river water added 10 ppm of MDI



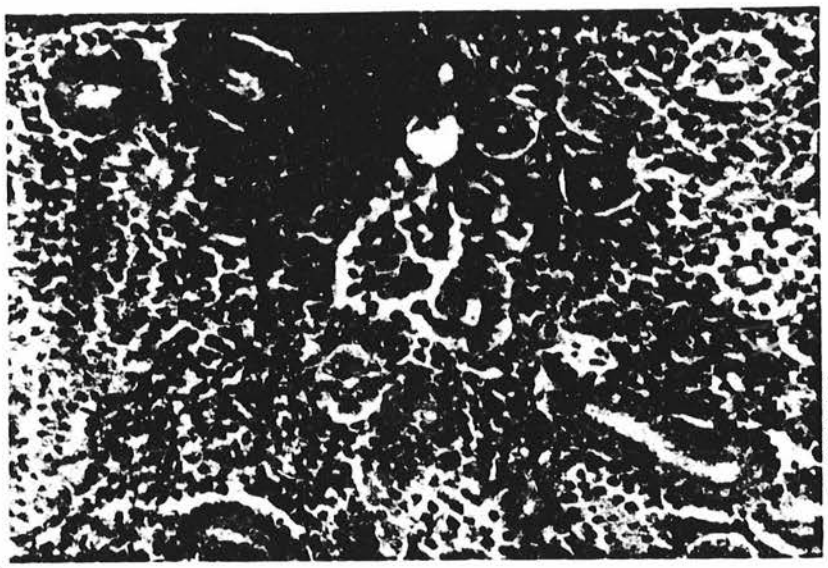
Photograph 4 --- Liver tissue of carp control



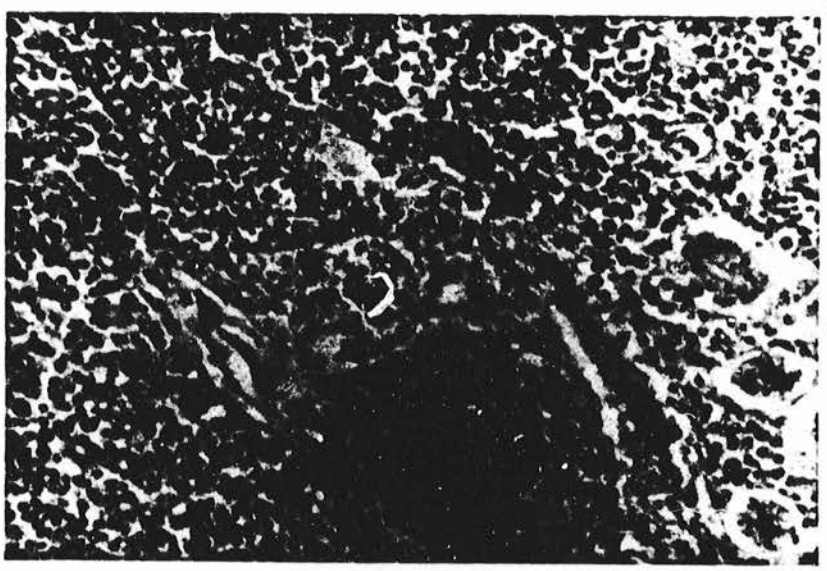

Photograph 5 —— Liver tissue of carp lived for 35 days in liver water added 10 ppm of TDI



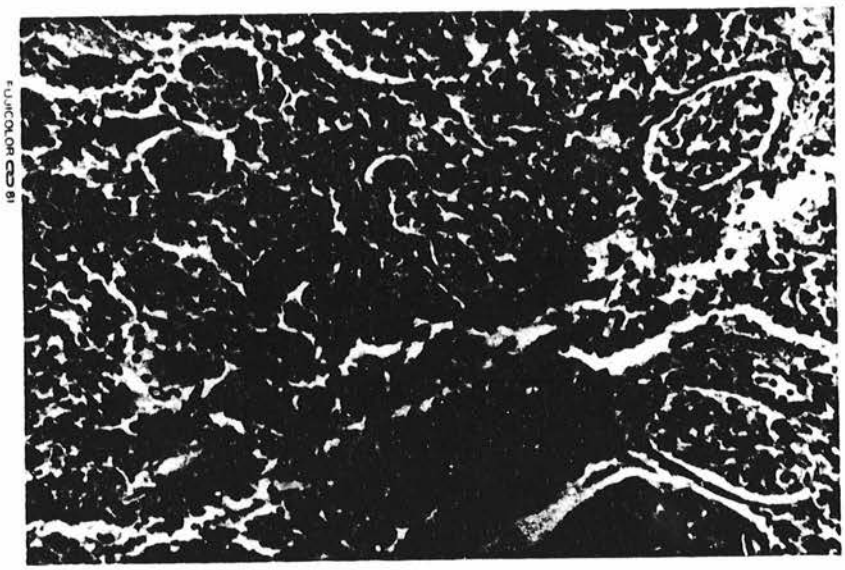

Photograph 6 —— Liver tissue of carp lived for 35 days in river water added 10 ppm of MDI



Photograph 7 —— Kidney tissue of carp control



Photograph 8 —— Kidney tissue of carp lived for 35 days in river water added 10 ppm of TDI



Photograph 9 —— Kidney tissue of carp lived for 35 days in river water added 10 ppm of MDI .

Photograph 10 — Bone tissue of carp control


Photograph 11 — Bone tissue of carp lived for 35 days in river water added 10 ppm of TDI


Photograph 12 -- Bone tissue of carp lived for 35 days in river water added 10 ppm of MDI

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